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Oral Presentation

Enhanced protein expression through strain selection, gene disruption, improved vector design and co-expression of endogenous chaperones

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Backgound

The use of *Saccharomyces cerevisiae* as a host system has been limited by the perception of limited secretion capacity, unstable episomal vectors and aberrant glycosylation. Solutions to all of these limitations are now available.

Results

An analysis of a series of haploid laboratory yeast strains revealed significant intra-strain variability and unstable plasmid segregation. By combining classic chemical mutagenesis and selection a family of highly efficient *Saccharomyces cerevisiae* strains has been developed for the commercial production of biopharmaceutical products. When combined with a stable [1], high copy number [2], episomal expression vector system and a strong constitutive promoter, secreted recombinant protein expression titres in excess of 4 g/L were achieved (see Figure 1). Specific genetic modifications to the host were also introduced to increase product yield and control post-translational modifications, such as proteolysis and glycosylation.

The expression vectors have been further enhanced to facilitate the stable co-expression of multiple proteins. When one of these proteins is a chaperone, the titre of co-expressed recombinant transferrin was increased 15-fold. The applicability of this system has been demonstrated with a wide range of heterologous proteins and is scalable from 10 mL shake flask to cGMP manufacture at high cell density fermentation (8,000 L) in a defined synthetic medium; designed to be integrated with cost-efficient downstream processing.

Conclusion

Significant intra-strain variability and unstable episomal plasmid systems have limited the usefulness of *Saccharomyces cerevisiae* as an industrial host for the production of biopharmaceuticals. However co-enhancement of the episomal vector system and the host strains is not only possible but has led to significant improvements in recombinant protein production.

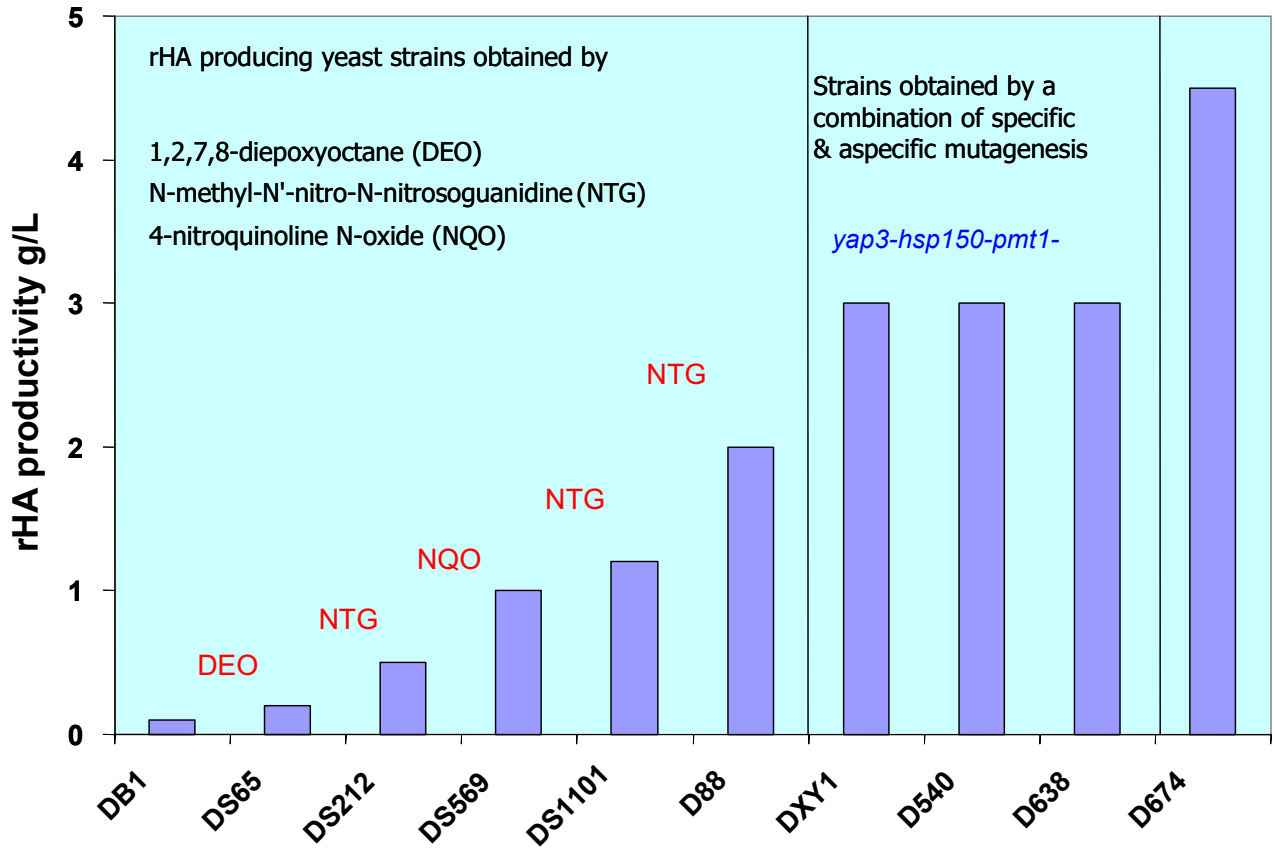

Figure 1
Enhancement of protein production through chemical mutagenesis and specific gene disruptions.

References

1. Chinery SA, Hincliffe E: **A novel class of vector for yeast transformation.** *Curr Genet* 1989, **16:**21-25.
2. Sleep D, Finnis C, Turner AJ, Evans LR: **Yeast 2 mm plasmid copy number is elevated by a mutation in the nuclear gene *UBC4*.** *Yeast* 2001, **18:**403-421.